REMARKS

Claims 11-14, 17-20 and 23-25 remain in the application. Claims 11 and 23 are the only independent claims pending.

The Office Action states that Applicant has not complied with the sequence rules. In order to further prosecution, attached hereto is a paper copy of the sequence listing, a computer readable form of the sequence listing, and a statement that the sequence listing does not contain any new material. Reconsideration of the rejection is respectfully requested.

Claims 11-14, 17-20 and 23-25 stand rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-9 of U.S. Patent No. 5,932,780. A terminal disclaimer is attached hereto, thereby rendering the present rejection moot. Reconsideration of the rejection is respectfully requested.

Claims 11-14, 17-20 and 23-25 stand rejected under 35 U.S.C. § 101 because the claims are drawn to non-statutory subject matter. The Office Action states that the rejection can be overcome by inserting "non-human" before "animal". In order to further prosecution, the claims have been amended to recite a "non-human animal" as suggested. Reconsideration of the rejection is respectfully requested.

Claims 11-14, 17-20 and 23-25 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

More specifically, the Office Action holds that the claims state that the transgene can be naturally occurring variants or derivatives of AChE and BChE, synthetic variants of AChE and BChE. The Office Action states that the specification does not provide for such variants or derivatives in their breadth to convey to the skilled artisan that at the time of the instant invention that Applicant had possession of the variants.

However, naturally occurring variants of both enzymes were readily available to one of skill in the art prior to the date of the application. For example, the detailed sequence specification of point mutations in AChE and BChE genes of human origin can be found in Ehrlich et al., Genomics 1994, which has been previously submitted. Other variants have also been reported in the literature, these include: the C-terminally truncated AChE-C, the construction and expression of which is described in Sternfeld et al. (J. Neurosci. 1998); the frog expressed BChE variants which is described in Loewenstein-Lichtenstein et al. (Mol. Pharmacol., 1996); AChE variants (Sternfeld et al., 1998, J. Neurosci., and J. Physiol., for frog tadpoles and

mice respectively). Accordingly, there is sufficient support either in the specification or in the prior art to enable spliced variants of AChE such that an individual of skill in the art would be able to practice the claimed invention.

Claims 11-14, 17-20 and 23-25 stand rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for transgenic mice and frog tadpoles whose genomes comprise a transgene comprising a AChE promoter operatively liked to a DNA sequence encoding a splice variant of human AChE expressing AChE with acetylcholine esterase activity, wherein the sequence is expressed in cells of the mouse and where the mouse or tadpole exhibits changes in its neuromuscular junction structure, and assay systems of the mouse or tadpole, does not provide enablement for the preparation and use of transgenic animals comprising any and all variants of the cholinesterase genes or assay system of these animals.

The Office Action states that with regard to the breadth of the claims, at the time of filing the art as a whole recognized that the production of transgenic animals as a whole was unpredictable. Additionally, the Office Action states that the specification does not provide guidance to an artisan at the time of the invention as to the promoter, vector construct, DNA sequence or additional expression regulatory sequences which correlates with the production of a transgenic non-human mammal expressing either human AChE or human BChE in its milk.

However, vectors have been prepared vectors which include the SIN variant with a seven amino acid insert in its active site. This protein variant maintains the structure and electrophoretic migration of its parent molecule.

The CMV promoter that was used by Applicants to direct the expression of the transgene has been tested by others and has been shown to be effective in the examined tissues. As described in the specification, in addition to the AChE promoter, also described is the CMV promoter. The CMV promoter is far more potent than the AChE promoter in frog tadpoles. It is ubiquitously expressed in numerous tissues and was used by others to derive efficient expression of C-myc in the mammary gland. As shown in Example 10, the CMV promoter was specifically tested in specific tissues and the native homologue of the transgene has been shown to faithfully produce its fully active protein products in these tissues. The combination of the CMV promoter and the transgenic variants has been tested in mice (Sternfeld et al, J. Physiol. 1998; Frog tadpoles; Ben-Aziz Aloya et al., PNAS 1993; Shapira et al., PNAS 1994; Seidman et al, McB 1995 and rat phaeochromocytoma cells Grifman et al., PNAS 1998; and C_6 glioma cells Karpel et al., J. Neurochem. 1996). Once proven active, consideration to this DNA methylation and deletion become irrelevant, as neither of these processes has apparently taken place in the examples set forth by the inventors.

With regard to lack of expression in other animals, this holds true only for those genes that are not naturally expressed in the animal, because in

that case it is not clear that the tissue in question would allow correct transcription, translation and post-translational modifications. Yet, for the exemplified CMV-AChE constructs, neither of these limitations exist. Applicants have shown correct sedimentation constants in sucrose gradient centrifugation for the catalytically active transgenic enzyme; correct electrophoretic migration in non-denaturing gel electrophoresis followed by cytochemical activity staining; and correct size as judged by fully denaturing gel electrophoresis followed by immuno-detection. As the claims are drawn to AChE and BChE variants, this is fully supported by the specification.

Additionally, the Office Action states that the AChE and BChE are "membrane anchored." This is inaccurate as AChE itself is a secretory, extracellular protein, the membrane and anchor of which is provided by a non-catalytically active structural subunit. In tissues that do not express such structural subunits, the enzyme will not adhere to the membrane. Also, glycosylation was proven experimentally not to be essential for the catalytic activity of AChE. In *E. coli*, a heterologous system that does not allow glycosylation, human AChE was produced with fully active properties. Further, the Office Action makes an assumption that "there is no readily apparent reason to produce the enzyme if it is not biologically active in hydrolyzing a choline ester bond." The assumption here is that each protein has one and only one function. However, the contrary has been established by the various applications filed by Dr. Soreq relating to both neurite growth promotion and the neuronal differentiation activity in progenitor cells shown to exist for the

proteins. Accordingly, there is sufficient support in the specification as filed for the claims as pending and reconsideration of the rejection is respectfully requested.

Claims 11-14, 17-20 and 23-25 stand rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly set forth and distinctly claim the subject matter which Applicant regards as the invention.

With regard to claims 11, 13, 20, and 23, the Office Action states that the phrases "normal human AChE", "normal human BChE", "synthetic variants of AChE and BChE" and "normal insect ChE's" make the metes and bounds of the claims unclear. The Office Action suggests that using the phrases "wild-type" instead of "normal", and the use of "variants" instead of "naturally occurring variants" or "synthetic variants" will overcome the present rejection. In order to further prosecution, the claims have been amended to make these changes. Reconsideration of the rejection is respectfully requested.

With regard to claims 13 and 18, the Office Action states that the phrase "substantial" and "substantially" make the claims unclear. Accordingly, in order to further prosecution, these words have been removed from the pending claims. Reconsideration of the rejection is respectfully requested.

With regard to claim 13, the Office Action states that the claim is

confusing because SEQ ID No: 20 is not an amino acid sequence. Accordingly, in order to further prosecution, the claim has been amended to clarify the confusion. Reconsideration of the rejection is respectfully requested.

With regard to claim 23, the Office Action states that the claim is confusing because it depends upon itself and there is no previous claim to a transgenic female. In order to further prosecution, the claim has been amended to clarify the confusion. Reconsideration of the rejection is respectfully requested.

With regard to claim 25, the Office Action states that the claim is confusing because there is no SEQ ID No: 28. In order to further prosecution, the sequence listing has been amended to include SEQ ID NO: 28 thereby clarifying the confusion. Reconsideration of the rejection is respectfully requested.

In conclusion, the application is in condition for allowance, which allowance is respectfully requested.

The Commissioner is authorized to charge any fee or credit any overpayment in connection with this communication to our Deposit Account No. 11-1449.

Respectfully submitted,

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CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231 on May 4, 2001.

Connie Herty

VERSION WITH MARKINGS TO SHOW CHANGES MADE

11. (Amended) A transgenic non-human animal carrying a recombinant DNA expression vector encoding a heterologous cholinesterase (ChE) enzyme selected from the group consisting of:

(a) [normal] wild-type human AChE;

(b) [normal] wild-type human BChE;

(c) [naturally-occurring] variants of the AChE and BChE of (a) and (b);

(d) [synthetic] variants of the AChE and BChE of (a) and (b), said synthetic variants selected from recombinantly-produced point-mutated and deletion of one or more residues, mutations; and

(e) [normal] wild-type insect ChEs, said transgenic animal being capable of expressing [substantial] amounts of said ChE enzyme for studying control of production on biochemical properties of cholinesterases.

Claim 12, line 1, before "animal" please insert --non-human--.

13. (Amended) A transgenic non-human animal according to claim 12, which carrying a recombinant expression vector encoding a human AChE or biologically active derivatives thereof selected from:

(a) a DNA sequence which has all or part of the nucleotide sequence (SEQ ID NO: 1) [substantially] as depicted in Figure 1A, and which encodes an amino acid sequence [substantially] similar or identical to all or part of the sequence of [amino] nucleic acid residues (SEQ ID NO: 20) depicted in Fig. 1B;

(b) a DNA sequence which has all or part of the nucleotide

sequences (SEQ ID NO: 3) [substantially] as depicted in Fig. 1C, and which encodes an amino acid sequence [substantially] similar or identical to all or part of the sequence of amino acid residues (SEQ ID NO: 4) also depicted in Fig. 1C; and

(c) A DNA sequence which has all or part of the nucleotide sequence (SEQ ID NO:5) [substantially] as depicted in Fig. 1D, and which encodes an amino acid sequence [substantially] similar or identical to all or part of the sequence of amino acid residues (SEQ ID NO: 6) also depicted in Fig. 1D.

Claim 14, line 1, before "animal" please insert --non-human--.

Claim 17, line 1, before "animal" please insert --non-human--.

Claim 18, line 1, before "animal" please insert --non-human--.

Claim 18, line 2, please delete "substantial".

Claim 19. (Amended) The transgenic <u>non-human</u> animal according to claim 18, wherein said ChE enzyme is selected from the group consisting essentially of [normal] <u>wild-type</u> human AChE, [naturally occurring] variants of AChE, and [synthetic] variants of the AChE.

20. (Amended) The transgenic <u>non-human</u> animal according to claim 19, wherein said [synthetic] variants are selected from the group consisting essentially of recombinantly-produced point mutation and deletion of one or more residues and mutations.

23. (Amended) A transgenic female <u>non-human</u> mammal [according to claim 23] wherein said ChE enzyme is selected from the group consisting of:

(i) [normal] wild-type human AChE;

(ii) [naturally occurring] variants of AChE [of claim 23]; and

(iii) [synthetic] variants of the AChE [of claim 23], said synthetic variants selected from recombinantly produced point mutated and deletion of one or more residues and mutations.

Claim 24, line 3, before "animal" please insert --non-human--.

Claim 24, line 4, please delete "or 24".

Claim 25, line 1, before "animal" please insert --non-human--.